

Characterization of Aroma-Active Compounds in Microwave Blanched Peanuts

A.V. SCHIRACK, M.A. DRAKE, T.H. SANDERS, AND K.P. SANDEEP

ABSTRACT: Microwave blanching of peanuts has been explored as an alternative to conventional oven methods based on its speed of operation, energy savings, and efficiency of process control. Although processing times can be greatly reduced, the occurrence of stale/floral and ashy off-flavors has been reported at high process temperatures. This study examined the chemical compounds responsible for this off-flavor using solvent extraction/solvent assisted flavor evaporation (SAFE), gas chromatography-olfactometry (GC/O), gas chromatography-mass spectrometry (GC/MS), and aroma extract dilution analysis (AEDA). Select compounds were quantified based on AEDA results using SAFE and GC/MS. Quantification, threshold testing, and analysis of model systems revealed increased formation of guaiacol and phenylacetaldehyde in the off-flavored peanuts, which resulted in the burnt and stale/floral flavors noted by a trained panel.

Keywords: gas chromatography-olfactometry, microwave, off-flavor, peanut, threshold

Introduction

The most common use of world peanut production remains the crushing of peanuts for oil and meal. However, the proportion of peanuts used for other food products has steadily increased. The unique flavor of roasted peanuts drives product marketing for products such as peanut butter and confections. This flavor is the result of genetics, production, handling, storage, and processing factors (Sanders and others 1995).

The main sources of volatile flavor compounds in peanuts are nonenzymatic carbonyl-amine browning and lipid oxidation reactions, and include interactions between peanut components as well as thermal decomposition products and loss of volatiles (Warner and others 1996). Maillard reactions are primarily responsible for browning reactions in roasted peanuts, and produce pyrazines, pyrroles, furans, and other low molecular weight compounds. In addition to Maillard products, carbonyls are produced by Strecker degradation and oxidation, but can then be lost by volatilization (Buckholz and others 1980). Pyrazines, which are volatile heterocyclic nitrogen-containing compounds, are thought to be the major flavor compounds impacting roasted peanut flavor (Warner and others 1996).

The causes of off-flavors in peanuts include lipid oxidation, induction of anaerobic respiration, and external contamination with compounds such as limonene, antioxidants, or insecticides (Ory and others 1992). Lipid oxidation is one of the leading causes of off-flavors in raw and roasted peanuts, due to a high content of unsaturated fatty acids (Warner and others 1996; Lee and others 2002). Oxidation of the fatty acids in peanut oil can be caused by light, heat, air, metal contamination, microorganisms, or enzymatic activity (Ory and others 1992; Sanders and others 1993). Hydroperoxides formed during lipid oxidation subsequently break down into alcohols, alkanes, ketones, and aldehydes, which can be the source of off-flavors in the peanut. High concentrations of certain compounds

such as ethanol, ethyl acetate, and acetaldehyde were found in high temperature cured peanuts (Pattee and others 1965). In addition, fruity fermented off-flavor has been shown to occur predominantly in immature peanuts undergoing high temperature curing (Sanders and others 1989; Didzbalis and others 2004).

Most previous studies examining the effects of processing techniques on peanut flavor have concentrated on high temperature curing. However, new processing technologies have been developed which can improve production efficiency but can also impact flavor quality. For example, microwave technology has been investigated as an alternative method for the drying and roasting of peanuts (Megahed 2001; Yoshida and others 2005). Although microwave roasting led to formation of undesirable lipid oxidation products, the use of microwaves for blanching has potential as an alternative to traditional blanching methods due to the speed of operation, energy savings, and efficiency of process control. However, during high temperature microwave treatments, an off-flavor has been observed which was related to other off-flavors such as cardboard, ashy, and bitter, and was related inversely to positive attributes such as roast peanutty and sweet aromatic (Schirack and others 2006).

The objective of this study was to investigate the off-flavor formed in peanuts during the high temperature heating step of microwave blanching through instrumental volatile analysis and model systems. The identification of the compounds responsible for the off-flavor could enable better quality control and may ultimately aid in the adoption of alternative blanching methods in peanut processing.

Materials and Methods

Peanuts

Medium-grade size, runner-type peanuts (*Arachis hypogaea* L., variety Georgia Green) at an average moisture content of 7% (wet basis) were obtained from a single harvested lot from USDA, ARS, Natl. Peanut Research Laboratory (Dawson, Georgia). The peanuts were harvested, cured, shelled, sized, and stored according to normal practices prior to delivery to Raleigh, N.C. Peanuts were heated as part of the blanching process using a 5 kW, 915 MHz microwave unit (Industrial Microwave Systems, Morrisville, N.C., U.S.A.) using

MS 20060340 Submitted 6/15/2006, Accepted 9/5/2006. Authors Schirack, Drake, and Sandeep are with Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27695-7624, U.S.A. Author Sanders is with USDA-ARS, Market Quality and Handling Research Unit, North Carolina State Univ., Raleigh, NC 27695-7624, U.S.A. Direct inquiries to author Drake (E-mail: mdrake@unity.ncsu.edu).

the equipment and methods detailed previously in Schirack and others (2006). A filled conveyor of peanuts (approximately 6 kg) was exposed to the microwave field for 11 min in a continuous process in which internal peanut temperatures were as high as 128 °C. Immediately after heating, peanuts were cooled to room temperature using forced ambient air. The control sample was peanuts undergoing the same preparation and storage procedures but not treated with microwave energy. The peanuts were roasted before descriptive sensory and instrumental analysis in order to approximate the impact of the off-flavor on commercial products such as confections and peanut butter. The peanuts were also roasted to avoid interference of the strong raw/beany note of unroasted peanuts with off-flavor detection (Didzbalis and others 2004).

An 800 g sample for each replicate was roasted and processed into paste for sensory and instrumental analysis. A thermostat-controlled Aeroglide Roaster was used (Aeroglide Corp., Raleigh, N.C., U.S.A.) to roast samples at 177 °C for the time needed to achieve *L* values in the range of 48 to 52 (Vercellotti and others 1992a) using a Hunter LAB DP-9000 colorimeter (Hunter Associates Laboratory, Reston, Va., U.S.A.). Samples were ground into paste using a food processor (Cuisinart Little Pro Plus, Cuisinart Corp., East Windsor, N.J., U.S.A.). A grind/cool protocol was used to prevent overheating of the paste, as discussed by Sanders and others (1989). Samples were kept frozen at –20 °C in glass jars until evaluation.

The peanut samples evaluated by instrumental analysis were selected based on sensory analysis results. For descriptive sensory analysis, samples were coded with 3 digit random codes, and evaluated against controls for each of 4 processing replications. The sensory panel consisted of 10 panelists, each with at least 40 h training in peanut sensory evaluation. Panelists were trained with the Spectrum™ Descriptive Analysis method using a 15-point intensity scale (Meilgaard and others 1999). Each sample was evaluated in duplicate by each panelist. Samples were described using the peanut lexicon developed by Johnsen and others (1988) and Sanders and others (1989), with the addition of some attributes identified by the trained panel for these samples, such as ashy, as defined by the aroma of cigarette ash; and total offnote, an attribute that encompassed all negative attributes which were different from the control. The 11-min microwave blanching treatment displayed the highest overall offnote and the highest ashy flavor by descriptive analysis (Table 1) (Schirack and others 2006). As a result, the 11-min blanching treatment sample and its process control were selected for instrumental volatile analyses.

Table 1 – Effect of high temperature microwave blanching on sensory attributes of roasted peanuts

Attribute	Process control	Microwave blanched peanuts
Roast peanutty	4.3a ^a	4.3a
Sweet aromatic	2.9a	2.8a
Dark roast	3.0a	3.3b ^b
Raw beany	2.1a	1.9a
Woody/hull/skins	3.1a	3.1a
Cardboardy/stale	0.61a	1.2b
Sweet taste	2.5a	2.5a
Bitter	3.3a	3.4b
Astringency	1.0a	1.0a
Ashy	0.5a	0.8b
Total offnote	1.2a	2.3b

^aAttribute intensities were scored using the 15-point Spectrum™ universal intensity scale (Meilgaard and others 1999).

^bMeans followed by different letters are significantly different between treatments (*P* < 0.05).

Chemicals

Ethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), 2-methyl-3-heptanone (internal standard for the neutral/basic fraction), and 2-methylvaleric acid (internal standard for the acidic fraction) were obtained from Sigma-Aldrich Corp. (St. Louis, Mo., U.S.A.). The standards for the aroma compounds listed in Table 3 were provided by the Sigma-Aldrich Corp. (St. Louis, Mo., U.S.A.) with the exception of tetradecanal (VWR, West Chester, Pa., U.S.A.).

Static headspace gas chromatography

Static headspace chromatography was conducted to screen the most volatile flavor compounds in the sample as possible contributors to the microwave-related off-flavor. Peanut samples were analyzed using 1 g of peanut paste in a 10 mL crimp-top vial. An external standard of hexanal diluted in acetone at 104 ppm was used. The sample was heated for 30 min at 150 °C. After heating, a carrier gas flow of 17 mL/min was used to sample the headspace for 0.5 min using a Turbomatrix 40 Headspace Sampler (Perkin Elmer Life and Analytical Sciences, Inc., Wellesley, Mass., U.S.A.). For separation and identification of headspace volatiles, a Perkin Elmer Autosystem XL gas chromatograph (GC) was coupled to a Perkin Elmer Turbomass Gold mass spectrometer (MS; Perkin Elmer Life and Analytical Sciences, Inc.). The injector temperature was maintained at 150 °C. Separations were performed on a fused silica capillary column (ZB-5, 30 m × 0.25 mm i.d., 1.0 μm d_f; Phenomenex, Torrance, Calif., U.S.A.). The GC oven temperature was programmed to increase from 35 °C to 300 °C at a rate of 15 °C/min with an initial and final hold time of 1 min each. The carrier gas was helium with a flow rate of 0.83 mL/min, and the flow was split at a 20 to 1 ratio. Mass spectrometer conditions were as follows: capillary direct interface temperature, 270 °C; ionization energy, 70 eV; mass range, 50 to 300 a.m.u; EM voltage (Atune + 306 V); scan rate, 0.5 scans/s. Each sample was evaluated in duplicate.

Solvent extraction with solvent assisted flavor evaporation (SAFE)

Compounds of a higher molecular weight were screened using a solvent extraction/SAFE technique to determine if they contribute to the microwave-related off-flavor. One hundred grams of peanut paste was weighed and placed in Teflon® bottles. Then, 100 mL of ethyl ether, 100 mL saturated sodium chloride solution, and 2.45 ppm of internal standard (comprised of 2-methyl-3-heptanone and 2-methyl pentanoic acid in methanol) were added. The mixtures were shaken for 30 min on a Roto mix (Type 50800; Thermolyne Dubuque, Iowa, U.S.A.) at high speed. The bottles were then centrifuged at 3000 rpm for 15 min in order to separate the solvent phase from the mixture, which was subsequently transferred to a glass jar. The procedure was repeated twice with the addition of 100 mL of ethyl ether to the sample each time.

Volatile compounds from the solvent extract were collected using solvent assisted flavor evaporation (SAFE). The assembly used was similar to that described by Engel and others (1999). Distillation was carried out for 2 h under vacuum (ca. 10^{–4} Torr). The sample was loaded into the top of the SAFE apparatus, and released into the vacuum dropwise. The SAFE apparatus was maintained at 50 °C with a circulating water bath. After distillation, the distillate was concentrated to 20 mL under a gentle stream of nitrogen gas.

The concentrated distillate was washed twice with 3 mL sodium bicarbonate (0.5 M) and vigorously shaken. It was then washed 3 times with 2 mL saturated sodium chloride solution. The ether layer containing the neutral/basic fraction was collected, dried over

anhydrous sodium sulfate, and concentrated to 0.5 mL under a gentle stream of nitrogen gas. Acidic volatiles were recovered by acidifying the aqueous phase with hydrochloric acid (18% w/v) to a pH of 2.0 and extracting the sample 3 times with 5 mL ethyl ether. The sample was dried over anhydrous sodium sulfate before being concentrated to 0.5 mL under a nitrogen gas stream.

Gas chromatography/olfactometry (GC/O)

For GC/O analysis, an HP5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif., U.S.A.) equipped with a flame ionization detector (FID), sniffing port, and a splitless injector was utilized. Both the neutral/basic and acidic fractions were analyzed from each extraction. Two microliters were injected onto a polar capillary column (DB-WAX, 30 m length \times 0.25 mm i.d. \times 0.25 μ m film thickness of stationary phase [d_f]; J. & W. Scientific) and a non-polar column (DB-5MS, 30 m length \times 0.25 mm i.d. \times 0.25 μ m d_f ; J. & W. Scientific). Column effluent was split 1:1 between the FID and sniffing port using deactivated fused silica capillaries (1 m length \times 0.25 mm i.d.). The GC oven temperature was programmed to increase from 40 °C to 200 °C at a rate of 8 °C/min with an initial hold for 3 min and a final hold of 20 min. The FID and sniffing port were maintained at a temperature of 250 °C. The sniffing port was supplied with humidified air at 30 mL/min.

Both post peak intensity and aroma extract dilution analysis (AEDA) were used to characterize the aroma properties and perceived intensities of the aroma-active compounds in the solvent extracts (Grosch 1993; Van Ruth 2001). Four experienced panelists with at least 40 h of training sniffed the neutral/basic and acidic fractions of the solvent extracts on the 2 different columns. For post peak intensity analysis, panelists described the odor and scored the intensity of odorants in the extracts using a 5-point numerical intensity scale (Van Ruth 2001). For AEDA, the solvent fractions were serially diluted at a ratio of 1:3 (v/v) with diethyl ether and sniffed (using a DB-WAX column for acidic fractions and a DB-5MS column for neutral basic fractions) until no odorants were detected by the panelists.

Gas chromatography/mass spectrometry (GC/MS)

For GC/MS analysis of the solvent extracts, a 6890N GC/5973 mass selective detector (Agilent Technologies, Inc., Palo Alto, Calif., U.S.A.) was used. Separations were performed on a fused silica capillary column (DB-5MS, 30 m length \times 0.25 mm i.d. \times 0.25 μ m d_f ; J. & W. Scientific). Helium gas was used as a carrier at a constant flow of 1 mL/min. Oven temperature was programmed to increase from 40 °C to 200 °C at a rate of 2 °C/min with initial and final hold times of 5 and 30 min, respectively. Mass selective detector conditions were as follows: capillary direct interface temperature, 250 °C; ionization energy, 70 eV; mass range, 50 to 300 a.m.u.; EM voltage (Atune + 200 V); scan rate, 2.94 scans/s. Each extract (1 μ L) was injected in duplicate in the splitless mode.

Identification of odorants

Retention indices (RI) were calculated using an n-alkane series (Van den Dool and Kratz 1963). For positive identifications, RI, mass spectra, and odor properties of unknowns were compared with those of standard compounds analyzed under identical conditions. Tentative identifications were based on comparing mass spectra of unknown compounds with those in the mass spectral database of the Natl. Inst. of Standards and Technology (NIST 2002) and by matching the RI values and odor properties of unknowns against published values in the Kovats retention indices located at <http://www.flavornet.org>.

Quantification of odorants

Relative abundance of compounds was calculated relative to the peak areas of 2-methyl-3-heptanone (for the neutral/basic fraction) or 2-methylvaleric acid (for the acidic fraction). In the cases when target flavor compounds coeluted with other peanut volatiles, an extracted ion search was used for quantification. For guaiacol (m/z 124 and 109), toluene (m/z 91), heptanal (m/z 96 and 114), tetradecanal (m/z 96 and 194), 2-phenylethylalcohol (m/z 91), 2-methylbutanal (m/z 86 and 56), and 1,4-butanediol (m/z 71 and 57), the specific ions in parenthesis were monitored during analysis. The response factors of selected compounds were determined by direct addition of known amounts of standards to odor-free water prior to solvent extraction and SAFE. Response factors for the compounds were calculated using a 5-point standard curve on a DB-5 column (DB-5MS, 30 m length \times 0.25 mm i.d. \times 0.25 μ m d_f) using GC/MS. The selected compounds were then quantified using the response factor and the peak area ratio of the compound to the internal standard.

Threshold testing

Orthonasal detection thresholds of acetophenone, phenylacetaldehyde, and 2,6-dimethylpyrazine (in oil) and toluene, acetophenone, and 2,6-dimethylpyrazine (in water) were determined using the forced choice ascending concentration series method of limits (ASTM practice E 679-91; ASTM 1992). Compounds were diluted in methanol (for the water threshold) or in vegetable oil (oil threshold) before addition to the matrix of either deodorized water or vegetable oil. Deodorized water was prepared by boiling deionized water to two-thirds of its volume. The vegetable oil (Wesson, ConAgra Foods, Omaha, Nebr., U.S.A.) was obtained at a local grocery store. The compound concentrations were serially diluted by a factor of 3 for each level in the threshold test, and a 7-level series was used. Blank samples in each set were adjusted with the same concentration of methanol to eliminate any bias due to the solvent used. Each 2-ounce sample cup (Sweetheart Cup Co., Inc., Owings Mills, Md., U.S.A.) was filled to 20 mL and allowed to equilibrate for 1 h before testing. All sample preparation and testing was done with the lights off to minimize compound degradation during this time. Each level in the series was presented in a randomized order.

Panelists were asked to choose the different sample out of a set of 3, and to indicate whether they were guessing. The individual best estimate threshold was calculated by taking the geometric mean of the last concentration, which was incorrect, and the 1st concentration that was correct with no further samples missed. The group threshold was calculated as the geometric mean of the individual best estimate thresholds. Thirty-five panelists were used. The panelist's degree of certainty was used to adjust the best estimate threshold according to the method in Lawless and others (2000).

Sensory evaluation of peanut models

Sensory analysis of model systems was conducted to further investigate the compounds responsible for the off-flavor caused by high temperature microwave blanching in peanuts. Flavor models were prepared from peanut paste, which was chosen based on absence of off-flavor. The peanut paste was divided into 15 g portions, and the compounds were introduced by a disposable pipet. After addition of the chemicals, the peanut paste was stirred for 30 s and then equilibrated for 2 h prior to sensory analysis.

Phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine were prepared in methanol for aroma evaluation or in 95% ethanol for flavor evaluation across the concentration range found in the peanut samples by quantification (Table 2). The peanut models were evaluated in duplicate for aroma or flavor by 6 highly trained panelists, each with > 150 h of training in the sensory evaluation of peanuts.

Statistical analysis

Data were evaluated by analysis of variance using the general linear models procedure of SAS (v 9.1, Cary, N.C., U.S.A). Fisher's least significant difference (LSD) was used as a posthoc test.

Results and Discussion

Sensory analysis

The sensory attributes of high-temperature microwave-blanched peanuts were described previously (Table 1) by a descriptive sensory panel (Schirack and others 2006). Peanuts which had been microwave blanched were significantly higher ($P < 0.05$) in total offnote, which is a term encompassing all negative aspects of the sample that are different from a reference. The total offnote term was introduced to the current peanut lexicon (Johnsen and others 1988; Sanders and others 1989) for this study, because the descriptive panel had some difficulty in agreeing to the exact nature of the off-flavor. Based on the other attribute scores that were significantly higher ($P < 0.05$) than the process control, the microwave blanched peanuts also displayed higher intensities of dark/ashy, bitter, and cardboardy/stale notes, which also may contribute in part to the total offnote score.

Further descriptive panels were conducted with experienced panelists to more fully describe the nature of the off-flavor. Over the course of 5 sessions, the panelists agreed that the distinct off-flavor of microwave blanched peanuts (which had an average total offnote score of 2.3 on a 15-point intensity scale) was best characterized by the attributes of stale/floral, cardboardy, and burnt/ashy. Product references such as cigarette ash for the burnt/ashy attribute were very useful, although the development of clear chemical anchors would be even more beneficial in further clarifying this total offnote attribute to panelists.

Static headspace analysis

Static headspace analysis was conducted as the 1st step to screen the samples for compounds contributing to the microwave-related off-flavor. In this analysis, no unique volatile compounds were found in the off-flavored sample, which were not present in the process

control (data not shown). This technique did isolate compounds that have been previously identified with flavor deterioration in high temperature-cured peanuts such as hexanal, 3-methylbutanal, and 2-methylpentanal (Pattee and others 1965). However, the compound concentrations in the control and off-flavored samples were not significantly different ($P < 0.05$). Most compounds that are similar in volatility to hexanal can be lost during roasting (Ory and others 1992). In addition, this extraction technique isolates only the most volatile and lowest molecular weight flavor compounds. This could explain why flavor differences detected in roasted peanuts by the sensory panel were not reflected in static headspace results. As a result, the static headspace method was deemed not suitable in differentiating the microwave blanched samples from the control peanuts and was not investigated further.

Gas chromatography-olfactometry

Over 200 aroma-active compounds were detected through gas chromatography-olfactometry (GC/O) in the peanut samples, which is consistent with reviews of the flavor compounds in peanuts in the literature (Pattee and Singleton 1981). Although many flavor compounds have been documented in peanuts, few systematic studies of the relative importance and balance of the flavor compounds in peanuts have been conducted in recent years. In this study, aroma extract dilution analysis (AEDA) was used to narrow the list of compounds that may have the most impact on the flavor. In AEDA, solvent extracts were serially diluted by a factor of 3 until no odorants were detected by the panelists. The compounds with dilution factors (FD) greater than 5 for the process control and the off-flavored peanuts are shown for both the neutral/basic and acidic fractions (Table 3). Of the 38 compounds with the highest FD values, 26 were positively identified using odor properties, retention indices, and mass spectra; 10 were tentatively identified using odor properties and retention indices in comparison to standards; and 2 compounds remained unidentified.

Maillard reaction products and lipid oxidation products are known to affect peanut flavor. The impact of pyrazines, which have long been associated with the characteristic flavors of peanuts (Mason and Johnson 1966; Johnson and others 1971), was both increased and lessened in the microwave blanched samples. For example, the FD factor of 2,5-dimethyl-3-ethylpyrazine (brothy) was lower in the off-flavored samples, while 2,6-dimethylpyrazine (nutty/earthy) and 2-ethyl-5-methylpyrazine (fruity) FD factors were higher. Lipid oxidation compounds such as (E,E)-2,4-decadienal (fried/oxidized), (E,Z)-2,4-heptadienal (fatty), nonanal (green/floral), decanal (fried), and heptanal (fatty) were found in both the control and off-flavored peanuts. Products such as nonanal and decanal are formed from monohydroperoxide precursors during linoleate oxidation (Min and Smouse 1989). While some of these compounds such as heptanal are associated with cardboard or rancid off-flavors (Warner and others 1996), other lipid oxidation compounds such as hexanal and 2,4-decadienal have been documented in good quality peanuts (Vercellotti and others 1992b). Based on AEDA results, the role of lipid oxidation compounds in microwave-related off-flavor was not clear.

GC/O results correlate with quantitative differences best when olfactometry differences between samples are high (Cullere and others 2004). Seventeen compounds had the largest differences in AEDA results between the process control and microwave-blanched peanuts (that is, differences in FD factors of 3 or more). These compounds included floral compounds such as phenylacetaldehyde (rosy) and geranyl buyrate (rosy); fatty compounds such as (E,E)-2,4-decadienal (fried/oxidized), (E,Z)-2,4-heptadienal (fatty), and (E)-2-hexenoic acid (fatty); sweet or fruity compounds such as

Table 2—Model system concentrations added to reference peanut paste

Model Reference	Compound added	Concentration (ppb) ^a
1	2,6-dimethylpyrazine	1.64×10^4
2	2,6-dimethylpyrazine	1.77×10^4
3	2,6-dimethylpyrazine	1.90×10^4
4	Guaiacol	1.36×10^1
5	Guaiacol	1.83×10^1
6	Guaiacol	2.30×10^1
7	Phenylacetaldehyde	3.24×10^3
8	Phenylacetaldehyde	3.92×10^3
9	Phenylacetaldehyde	4.59×10^3
10	2,6-dimethylpyrazine	1.64×10^4
	Guaiacol	1.36×10^1
	Phenylacetaldehyde	3.24×10^3
11	2,6-dimethylpyrazine	1.77×10^4
	Guaiacol	1.83×10^1
	Phenylacetaldehyde	3.92×10^3
12	2,6-dimethylpyrazine	1.90×10^4
	Guaiacol	2.30×10^1
	Phenylacetaldehyde	4.59×10^3

^aConcentrations calculated based on average, average + σ , average + 2σ as determined in quantification results.

4-ethylbenzaldehyde (burnt sugar), benzaldehyde (sweet/malty), toluene (sweet/chemical), 2,3-butanediol (fruity), tetradecanal (honey/hay), methyl cinnamate (strawberry), 2-methylbutanal (chocolate/malty), and 2-ethyl-5-methylpyrazine (sweet/fruity); savory compounds such as 2,6-dimethylpyrazine (nutty/earthy) and 2,5-dimethyl-3-ethylpyrazine (brothy); and others such as guaiacol (burnt/smoky), and delta-elemene (wood). Many of these compounds have been reported previously in peanuts (Johnson and others 1971; Clark and Nursten 1977; Vercellotti and others 1992a). Specifically, several of these compounds have been associated with off-flavors in peanuts; 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, phenylacetaldehyde, and guaiacol (2-methoxyphenol) were identified in high temperature cured peanuts by Didzbalis and others (2004).

It is important to note that AEDA is only a semiquantitative technique, and it does not establish that compounds are present in concentrations above sensory threshold. AEDA also does not reflect the impact of the food matrix on the perception and odor properties of a compound. In fact, although the FD factors are relative to the

compounds' concentration in the extract, they are not measures for perceived odor intensity (Grosch 1993). No compound in the AEDA results by itself gave the exact odor noted in microwave-blanched peanuts. This indicated that the microwave-related off-flavor may be influenced by the other compounds in the food matrix or caused by a combination of compounds that are present in both samples, but at different concentration levels.

In order to compare volatile concentrations across samples, the relative abundances of compounds identified by GC/O were calculated using relative abundance: $\{(\text{peak area of internal standard} / \text{concentration of internal standard}) = (\text{peak area of compound} / \text{concentration of compound})\}$. The relative abundance values for compounds that were not further quantified are seen in Table 4. Many of the compounds in the acid fractions of the solvent extract were not different in flavor dilution factors, nor did they possess a unique character that could potentially contribute to the microwave-related off-flavor. Many of these compounds had a sweet or burnt sugar odor, which can be expected from Mailard reaction products. An examination of the relative abundances

Table 3 – High impact aroma-active compounds in peanuts as determined by AEDA

Nr	Compound	Fraction ^a	Odor ^b	RI ^c		Log ₃ FD factors ^d		Method of identification
				DB-5MS	DB-WAX	Control	Off-flavor	
1	2-methylbutanal	NB	Chocolate/malty	653	907	6	9	RI, odor, MS ^e
2	Toluene	NB	Sweet/chemical	756	1027	5	11	RI, odor, MS
3	2,3-butanediol	NB	Fruity	803	1554	3	9	RI, odor ^f
4	Furfural	AC	Sweet	821	1468	5	7	RI, odor, MS
5	(E)-2-hexenal	AC	Fruity	844	1188	3	5	RI, odor, MS
6	Ethyl valerate	AC	Fruity	915	1116	6	6	RI, odor ^g
7	2,6-dimethylpyrazine	NB	Nutty/earthy	934	1314	6	9	RI, odor, MS
8	Heptanal	NB	Fatty	937	1163	5	7	RI, odor, MS
9	(E,Z)-2,4-heptadienal	NB	Fatty	968	1399	<1	7	RI, odor, MS
10	2-ethyl-5-methylpyrazine	AC	Sweet/fruity	981	1323	4	7	RI, odor, MS
11	Methyl hexanoate	AC	Sweet	1015	1154	5	7	RI, odor, MS
12	Furaneol TM (2,5-dimethyl-4-hydroxy-3(2H)-furanone)	AC	Burnt sugar	1047	2049	8	7	RI, odor, MS
13	Phenylacetaldehyde	NB	Rosy/green	1058	1605	7	11	RI, odor, MS
14	Acetophenone	NB	Fruity/sweet	1080	1638	7	7	RI, odor, MS
15	Guaiacol	NB	Burnt	1089	1825	3	9	RI, odor, MS
16	2,5-dimethyl-3-ethylpyrazine	AC	Brothy	1091	1416	7	4	RI, odor, MS
17	2-ethyl-3,5-dimethylpyrazine	NB	Nutty/roasted	1095	1443	8	8	RI, odor, MS
18	Maltol (3-hydroxy-2-methyl-4H-pyran-4-one)	AC	Cotton candy	1106	1936	6	5	RI, odor, MS
19	2,3-diethyl-5-methylpyrazine	NB	Roasted	1153	1504	6	6	RI, odor, MS
20	Nonanal	NB	Green/floral	1159	1381	8	8	RI, odor, MS
21	4-ethylbenzaldehyde	AC	Burnt sugar	1163	1730	3	7	RI, odor
22	3-ethylphenol	NB	Old books/musty	1176	ND ^f	6	8	RI, odor, MS
23	3,5-diethyl-2-methylpyrazine	NB	Roasted	1184	ND	6	7	RI, odor, MS
24	Decanal	NB	Fried	1231	1485	4	3	RI, odor, MS
25	(E,E)-2,4-decadienal	NB	Fried/oxidized	1343	1740	7	4	RI, odor, MS
26	Decanoic acid	NB	Oxidized	1357	ND	7	8	RI, odor, MS
27	Delta-elemene	NB	Wood	1361	ND	6	1	RI, odor
28	4-acetoxy-2,5-dimethyl-3(2H)-furanone	AC	Burnt sugar	1386	1981	7	6	RI, odor
29	Delta-decalactone	AC	Sweet/fruity	1471	2209	5	7	RI, odor
30	Geranyl butyrate	NB	Rosy	1544	1888	3	8	RI, odor
31	Tetradecanal	NB	Honey/hay	1618	1931	6	2	RI, odor, MS
32	(E)-2-hexenoic acid	NB	Fatty	1632	1938	6	10	RI, odor
33	Pantolactone	AC	Burnt sugar	1689	1998	6	5	RI, odor, MS
34	Unknown	AC	Sweet	N/A	352	5	6	Odor
35	Unknown	AC	Sweet/malty	N/A	707	6	7	Odor
36	Benzaldehyde	AC	Sweet/malty	ND	1500	6	2	RI, odor, MS
37	Methyl cinnamate	AC	Strawberry	ND	2045	7	ND	RI, odor
38	3-methoxy-2,5-dimethylpyrazine	AC	Spicy/pepper	ND	1385	4	5	RI, odor

^aNB = neutral/basic; AC = acid.

^bOdor description by GC/O.

^cRetention indices (RI) were calculated from GC/O data.

^dFlavor dilution factors were determined on a DB-5MS column for neutral and basic compounds, and on a DB-WAX column for acidic compounds.

^eCompound identified by RI, MS data and odor character in comparison with the standard.

^fCompound tentatively identified using RI data and odor character in comparison with standard.

^gND: not detected.

revealed compounds that were below reported thresholds or which had no consistent differences between samples for this set of compounds.

Quantification

Select compounds were quantified by analysis of standards in deodorized water using solvent extraction, SAFE, and GC-MS analysis. Compounds were chosen for further quantification if they had large differences in AEDA results between the off-flavored peanuts and the control, or if they had been tied to off-flavors in the peanut literature (that is, lipid oxidation compounds). A selection of pyrazines was also quantified to determine whether these decreased in concentration in the off-flavored peanuts, because coincident decreases in the roasted peanut attribute have been documented with other off-flavors in peanuts (Sanders and others 1989; Didzbalis and others 2004). The 9 compounds selected for quan-

tification included 1 compound possibly contributing to the burnt note in the off-flavored peanuts (guaiacol), a compound possibly adding the stale/floral attribute noted by the sensory panel (phenylacetaldehyde), 2 pyrazines (2,6-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine), 2 compounds with sweet odors (acetophenone, toluene), and 3 lipid oxidation compounds (nonanal, decanal, 2,4-decadienal). A 5-point standard curve was used, and for all compounds, the linear fit had an $R^2 \geq 0.92$.

The results of quantification (Table 5) support the descriptive panel comments used to describe the off-flavor. The microwave-blanched peanuts were described as being more burnt/ashy, which could be due to an increase in guaiacol, and more stale/floral, which could be due to the increase in phenylacetaldehyde. The samples were not differentiated in levels of acetophenone or nonanal. Although large FD differences were seen between the samples for toluene, quantification results did not support these differences, but

Table 4—Relative abundance of selected high aroma impact compounds in peanuts

Compound	RI on DB-5MS ^b	Concentration in control (ppb) ^a	Concentration in off-flavored peanuts (ppb)	Threshold in water (ppb)	Threshold in oil (ppb)
Decanoic acid	1357	25.7 ± 18.6	48.2 ± 61.3	10000 ^d	Not reported
2-methylbutanal	653	2613 ± 856	4024 ± 789	1 ^d	2.2 ^d
Heptanal	937	0.41 ± 0.03	0.14 ± 0.04	3 ^d	250 ^d
(E,Z)-2,4-heptadienal	968	ND ^e	0.29 ± 0.05	Not reported	4000 ^d
2-ethyl-3,5-dimethylpyrazine	1095	5534 ± 3117	6961 ± 495	0.04 ^d	2.2 ^d
3-ethylphenol	1176	14.9 ± 4.5	16.5 ± 3.1	0.05 ^f	Not reported
3,5-diethyl-2-methylpyrazine	1184	554 ± 410	572 ± 28	Not reported	Not reported
Tetradecanal	1618	3.05 ± 1.98	0.63 ± 0.18	Not reported	Not reported
Compound	RI on DB-Wax ^c				
Methyl hexanoate	1142	486 ± 471	72 ± 67	50 ^d	Not reported
(E)-2-hexenal	1188	77 ± 48	15 ± 11	17 ^d	424 ^d
2-ethyl-5-methylpyrazine	1323	3441 ± 1937	498 ± 149	100 ^h	Not reported
2,5-dimethyl-3-ethylpyrazine	1416	352 ± 163	1239 ± 806	0.4 ^d	24 ^d
Furfural	1468	941 ± 514	536 ± 370	3000 ^d	Not reported
Benzaldehyde	1500	506 ± 250	328 ± 285	Not reported	Not reported
Maltol (hydroxymethylpyrone)	1936	303 ± 92	71 ± 59	210 ^g	Not reported
Pantolactone	1998	133 ± 44	126 ± 106	Not reported	Not reported
Furaneol TM	2049	59 ± 52	17 ± 13	0.6 ^d	25 ^d

^aAverage concentration ± standard deviation.

^bRetention indices (RI) were calculated from mass spectrometry results on a DB-5MS column.

^cRI calculated from flame ionization results on a DB-WAX column.

^dOrthonasal threshold reported by Rychlik and others (1998).

^eND: not detected.

^fRetronasal threshold reported by Rychlik and others (1998).

^gOrthonasal threshold reported by Karagul-Yuceer and others (2004).

^hOrthonasal threshold reported by Maga (1982).

Table 5—Quantification, sensory orthonasal threshold values, and odor activity values of selected compounds in peanuts

Nr	Compounds	RI on DB-5MS column ^a	Concentration in control (ppb)	Concentration in off-flavored peanuts (ppb)	Threshold in water (ppb)	Threshold in oil (ppb)	OAV of control using water threshold ^b	OAV of control using oil threshold	OAV of off-flavored peanuts using water threshold	OAV of off-flavored peanuts using oil threshold
1	Toluene	756	104 ± 30	114 ± 23	527 ± 4 ^c	94660 ^c	0.2	0.001	0.2	0.001
2	2,6-dimethylpyrazine	934	15234 ± 2594	40009 ± 2773 ^g	718 ± 5 ^c	1021 ± 3 ^c	21	15	56	39
3	Phenylacetaldehyde	1058	4447 ± 1894	8266 ± 1505 ^f	2 ^d	154 ± 4 ^c	2224	29	4133	54
4	Acetophenone	1080	3.60 ± 0.16	3.2 ± 3.2	245 ± 6 ^c	5629 ± 6 ^c	0.015	0.001	0.01	0.0006
5	Guaiacol	1089	13.7 ± 0.6	29 ± 5 ^f	2.5 ^e	16 ^e	5.5	0.9	12	1.81
6	2,3-diethyl-5-methylpyrazine	1148	2.2 ± 0.5	1.6 ± 0.3	0.09 ^e	0.5 ^e	24	4	18	3.2
7	Nonanal	1159	121 ± 79	168 ± 42	1 ^e	1000 ^e	121	0.1	168	0.17
8	Decanal	1231	3.7 ± 0.7	5.9 ± 0.5	0.1 ^e	6700 ^e	37	0.001	59	0.001
9	(E,E)-2,4-decadienal	1343	135 ± 85	28.9 ± 4.5	0.07 ^e	180 ^e	1929	0.8	413	0.16

^aRetention indices calculated from mass spectrometry results on a DB-5MS column.

^bThe odor activity value (OAV) is the ratio of the concentration to the threshold value of the compound.

^cOrthonasal threshold experimentally determined from 35 panelists.

^dOrthonasal threshold reported by Carunchia Whetstone and others (2005).

^eOrthonasal threshold reported by Rychlik and others (1998).

^fConcentration is significantly different from the control at $P < 0.05$.

^gConcentration is significantly different from the control at $P < 0.1$.

the AEDA differences may have been complicated due to coelution with the solvent peak during GC/O.

Threshold determination

In order to clarify quantification results, threshold analyses were conducted to gauge human perception of these compounds. Detection threshold values for the quantified compounds that were not available in the literature were determined experimentally using the ASTM ascending forced choice method of limits procedure (Table 5). Because peanuts are composed of approximately 50% fat, both the water and oil thresholds were evaluated. Based on these threshold values, guaiacol, phenylacetaldehyde, 2,6-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine had the most impact on the flavor of these samples. Phenylacetaldehyde, 2,6-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine concentrations in both control and off-flavored samples were above the threshold values. Not only were guaiacol concentrations in the off-flavored peanuts double that of the control, but only in the off-flavored peanuts did the concentrations exceed the compound's threshold in oil. Toluene, acetophenone, nonanal, decanal, and (E,E)-2,4-decadienal values were below the threshold values, either in the oil matrix or in both matrices.

After threshold testing, the odor activity value (OAV) of each compound in different matrices was determined in the control and microwave-blanched peanuts (Table 5). The OAV is the ratio of the compound concentration in a food to its sensory threshold. The OAV can further identify those compounds having the most flavor impact (Guth and Grosch 1994). In Emmentaler cheese, a high fat food, the oil threshold value was chosen to calculate OAV for evaluation of key compounds because the lipid phase predominated in the samples (Preininger and Grosch 1994). Similarly in this study, the OAVs in oil were compared due to the high lipid content of peanuts. Phenylacetaldehyde, 2,6-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, and guaiacol had the highest OAV in oil of the compounds quantified. The OAV values of phenylacetaldehyde, 2,6-dimethylpyrazine, and guaiacol were the highest in the off-flavored samples and were also approximately twice their OAV values in the control, which further supported the role of these compounds in the flavor profile of microwave-blanched peanuts.

Phenylacetaldehyde has been previously found in peanuts (Mason and others, 1967), in lavender honey (Bouseta and others 1996), and in other foods such as chocolate (Schieberle and Pfner 1999). Phenylacetaldehyde has also been linked to off-flavors, such as aroma deterioration in beer (Soares da Costa and others 2004) and rosy off-flavor in cheddar cheese (Carunchia Whetstone and others 2005). Phenylacetaldehyde is known to be generated in peanuts from phenylalanine through Strecker degradation (Mason and others 1967). Phenylalanine is typically present as a flavor precursor in peanuts and makes up a significant portion of the free amino acids present (Newell and others 1967). Guaiacol is found in strongly flavored cheeses (Suriyaphan and others 2001). This phenolic compound has also caused medicinal or antiseptic off-flavors in apple juice (Orr and others 2000). 2,3-Diethyl-5-methylpyrazine and 2,6-dimethylpyrazine have been correlated to peanut flavor (Mason and Johnson 1966; Maga 1982), and 2,3-diethyl-5-methylpyrazine is a key odorant in bitter chocolate (Schieberle and Pfner 1999).

Among these 4 key compounds, phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine were present at significantly different ($P < 0.10$) levels in the off-flavored samples, and as a result were pursued as the possible source of the microwave-related off-flavor. These 3 compounds are affected by increased temperatures. Pyrazine formation begins above 100 °C, and yield increases as the temperature increases (Koehler and Odell 1970). Although guaiacol can be produced by *Alicyclobacillus* spoilage (Orr and others 2000) and has

been associated with the maturation of wine in oak barrels (Pollnitz and others 2004), most pertinently to peanut production, guaiacol is also a thermal degradation product of ferulic acid during the roasting process (Holscher and Steinhart 1994). Similarly, the kinetic rate of phenylacetaldehyde formation was significantly increased with increasing temperatures (Soares da Costa and others 2004). During peanut blanching, the microwave process temperatures reached up to 128 °C, which may be high enough for pyrazine formation and could explain the increased formation of phenylacetaldehyde and guaiacol.

Interestingly, lipid oxidation compounds did not appear to have a role in microwave-related off-flavor. This is consistent with the literature, as Katz (2002) found that microwave-blanched peanuts were more oxidation stable than oven-blanched peanuts as evident by lower peroxide values and higher oxidative stability index. In addition, Maillard reaction products in peanuts such as reductones are free radical scavengers, which could further prevent formation of oxidation products (Sanders and others 1993).

Model systems

In order to examine the effects of these compounds at their relative concentrations in a food matrix, phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine were added singly and in combination to a freshly roasted peanut paste free of off-flavors (Table 2). Although these compounds individually had distinct aromas during GC/O of rosy (phenylacetaldehyde), smoky/burnt (guaiacol), and nutty/earthy (2,6-dimethylpyrazine), the flavor profile of the reference paste changed in different ways upon compound addition, emphasizing the effect of compound concentration and the effect of other components in the matrix.

In aroma evaluation, 6 out of 6 panelists agreed that the addition of phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine singly at the average concentrations found during quantification created notable differences from the control. In each of these models, a decrease in roasted peanutty aroma was observed. The addition of phenylacetaldehyde caused a green/plant-like note, while the addition of guaiacol gave a darker roast character to the model as compared to the control. 2,6-Dimethylpyrazine, although adding a sweet, caramel note at lower concentrations, became perceived as a sweet and rotten aroma at higher concentrations. In the tasting models, phenylacetaldehyde added a green/plant-like note at low concentrations, but created a stale/cardboard character at higher concentrations. Guaiacol added astringency, bitterness, and more ashy and woody character to the flavor. 2,6-Dimethylpyrazine added rotten notes to the flavor, and also contributed to the perception of dark roast flavor. A combination of these 3 compounds at their respective concentrations found in microwave blanched peanuts created an aroma profile high in dark roast character, with more astringency and tongue and throat burn, and less impact of positive characteristics such as roasted peanutty attribute. The panel agreed that the combination of phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine each at a concentration of 1 standard deviation above the average concentration found in the microwave-blanched samples appeared to most closely mimic the off-flavor in microwave-blanched peanuts.

The unique characters of these 3 compounds combine to form an off-flavor, which is difficult to define. Further work must be conducted to clarify the role of 2,6-dimethylpyrazine. However, it appears that guaiacol contributes to the dark roast/burnt flavor perceived in the microwave samples, and phenylacetaldehyde is responsible for a green and cardboard note, which could be perceived as stale/floral. In the future, these compounds could be used as chemical anchors for sensory panelists analyzing process

samples and would aid in the identification of process-related off-flavors.

Conclusion

More than 200 aroma-active compounds contributed to the flavor of roasted peanuts. Maillard reaction, lipid oxidation, and thermal degradation products dominated the flavor profiles. Isolation of the compounds causing a microwave-related off-flavor in peanuts was possible through solvent extraction/SAFE, GC/O, GC/MS, threshold testing and model systems analysis. The stale/floral and ashy off-flavor in microwave-blanched peanuts was related to increased concentrations of phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine. Increased and unfavorable levels of these compounds may have been formed through Maillard reactions and thermal degradation during the high temperatures attained during microwave blanching. These findings are important because they further explore the relative balance of the many aroma-active compounds, which have been documented in peanuts, and could possibly aid in enhancing quality control for alternative processing techniques in peanut production.

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